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Glomerulopathy Associated With Predominant Fibronectin Deposits: Exclusion of the Genes for Fibronectin, Villin and Desmin as Causative Genes

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Glomerulopathy with predominant fibronectin deposits (GFD) is a newly recognized autosomal dominant renal disease that leads to albuminuria, microscopic hematuria, hypertension, renal tubular acidosis type IV, and end-stage renal disease in the second to fourth decade of life. Light microscopy documents extensive deposits in the subendothelial space, which on electron microscopy consist of non-oriented 12 × 125 nm fibers. Deposits are strongly immunoreactive for antibodies to fibronectin. We examined the hypothesis that a genetic defect in the gene for fibronectin is responsible for the disease. In a 197 member pedigree, 13 relatives developed end-stage renal failure from the disease. In 99 individuals haplotype analysis was performed using 6 microsatellite markers spanning a >56 cM interval in chromosome region 2q34, where fibronectin, villin, and desmin map in close proximity. Haplotype analysis resulted in exclusion of the whole range of 78 cM covered by the markers examined. This result excludes fibronectin, villin, and desmin from being the causative genes for GFD in this large kindred. © 1996 Wiley-Liss, Inc.

KEY WORDS: glomerulopathy, fibronectin, haplotype analysis

INTRODUCTION

Glomerulopathy with predominant fibronectin deposits (GFD) is a newly recognized autosomal dominant disease [Strøm et al., 1995], which leads to albuminuria, microscopic hematuria, hypertension, renal tubular acidosis type IV, and end-stage renal disease in the second to fourth decade of life. While individual unrelated families with GFD have been reported previously [Abt et al., 1991; Bürgin et al., 1980; Mazzucco et al., 1992; Tuttle et al., 1987], the condition was recognized as a new disease by Strøm et al. [1995], following reevaluation of the histologic findings in all six families reported.

Light microscopy demonstrates enlarged glomeruli with minimal hypercellularity and giant fibrillary deposits in the mesangium and subendothelial space, without immunoreactivity for immunoglobulins or complement factors. On electron microscopy the deposits are mainly located in the subendothelial space, but also in the subepithelial and intramembranous spaces. The most striking finding in this disease is strong immune reactivity to fibronectin, corresponding to the distribution of the deposits [Strøm et al., 1995]. In all six families described, segregation was consistent with an autosomal dominant pattern of inheritance with age-related penetrance [Strøm et al., 1995]. This family was described previously with regard to its clinical [Bürgin et al., 1980; Gemperle et al., unpublished] and histologic characteristics [family F in Strøm et al., 1995].

Fibronectin is an adhesive high molecular weight glycoprotein, which is found in the extracellular matrix. It takes part in cellular proliferation, wound healing, and platelet aggregation and is present in the matrix of normal glomeruli. In the large kindred examined in this study deposits on electron microscopy were clearly fibrillary and consisted of non-oriented 12 × 125 nm fibers. The fibrils as seen on electron microscopy have been interpreted as deposition of fibronectin itself [Strøm et al., 1995]. In immunolocalization studies the deposits stained positively with an antibody detecting both, plasma-derived fibronectin and cellular fibronectin but were only weakly positive with an antibody detecting cellular fibronectin only. Notably, in one patient the fibronectin deposits recurred in a transplanted kidney, although there was no

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Dedicated to Jürgen W. Spranger on the occasion of his 65th birthday with admiration and best wishes.

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indication of systemic deposition of fibronectin. Taken together, these findings strongly suggested a molecular defect in circulating fibronectin.

Therefore we examined the hypothesis that a genetic defect in the gene for fibronectin is responsible for the disease. Haplotype analysis in 99 individuals of the 197 member pedigree (Gemperle et al., unpublished) was performed, using six microsatellite markers spanning a 78 cM interval in the chromosome region 2q34, where fibronectin, villin, and desmin map in close proximity. Since there was no cosegregation between the GFD affected status and haplotypes in this chromosomal region, the genes for fibronectin, villin, and desmin were excluded from being the causative genes for GFD.

METHODS

Patients

In a large 197 member GFD pedigree of five generations blood samples for isolation of genomic DNA were collected from 99 individuals with informed consent. As a result of extensive re-evaluation of the pedigree, this corrected pedigree structure differs slightly from the structure reported previously as family F by Strøm et al. [1995]. Thirteen relatives had developed end-stage renal failure from the disease. For the definition of the affected status by strict criteria, a biopsy sample compatible with GFD was required as described [Bürgin et al., 1980], which was available in nine of the affected individuals. In the other four individuals, who were not available for study, the clinical history was clearly compatible with an affected status. For the definition of unequivocal absence of disease the following inclusive list of criteria had to be met: 1) absence of proteinuria; 2) normal blood pressure (<140/90 mm Hg), adjusted for age in children; 3) normal renal function (plasma creatinine concentration below 1.0 mg/dl, and creatinine clearance >120 ml/min, adjusted for age in children); and 4) normal urine sediment. When microalbuminuria was detected, day time and overnight albuminuria was analysed to exclude orthostatic albuminuria. In one patient (III-3) (Fig. 2) the disease recurred in the renal allograft after transplantation, and another patient (III-11) developed organ-limited renal cell carcinoma as did his unaffected younger sister (III-18) (Gemperle et al., unpublished). The histological aspects of this large 197 family member kindred have been described previously [family F in Strøm et al. [1995]; Bürgin et al., 1980]. The initial clinical presentation in members of the pedigree was published by Bürgin et al. [1980], and the clinical course of 157 individuals of this kindred, 9 of whom were affected by GFD by strict criteria was communicated in a 15-year follow-up by Gemperle et al. (unpublished). Segregation of GFD in this pedigree is consistent with an autosomal dominant inheritance with age-related penetrance (Gemperle et al., unpublished).

Haplotype Analysis

Haplotype analysis was performed using six microsatellite DNA markers that span an interval of more than 56 cM (Fig. 1a) in the chromosome region 2q34, where fibronectin, villin, and desmin map in close proximity (Fig. 1). In Figure 1a comparison of four genetic maps is given, from which it is evident that marker D2S104 is a proximal and D2S172 is a distal marker to the fibronectin (FN1) gene locus. As an additional, even more proximal marker CTLA4, which maps to 2q33 [Spurr et al., 1994], was also included in haplotype analysis. Oligonucleotide sequences for microsatellite markers CTLA4, D2S104, D2S126, D2S102, D2S159, and D2S172 were from Spurr et al. [1994].

Oligonucleotides were synthesized with an Applied Biosystems Model 392 DNA/RNA synthesizer, and genomic DNA was extracted following standard techniques [Maniatis et al., 1987]. Genotyping with microsatellites was performed by radioactive multiplex PCR as described previously [Hildebrandt et al., 1993]. In brief, 30 ng of genomic DNA from individuals of the GFD kindred was used as template, 6–18 pmol of primers, 0.2 mM each of dATP, dGTP, dTTP, 2.5 μM dCTP, 0.1 μCi/μl ^{32}P-αdCTP, 10 mM Tris-HCl (pH7.3), 50 mM KCl, 0.001% gelatin (w/v), and 0.3 U of *Thermus aquaticus* DNA polymerase (Perkin Elmer Cetus, Überlingen, FRG). After initial denaturation at 94°C for 4 min 32 cycles were performed at 94°C for 30 s, 55°C for 40 s, and 72°C for 40 s, followed by a 6 min final extension step at 72°C. The amplified fragments were separated by electrophoresis in 8% denaturing polyacrylamide sequencing gels. The gels were blotted onto Whatman paper, dried, and autoradiography was performed for 2–16 hours. Genotypes were evaluated by two investigators independently.

RESULTS

Haplotype analysis was performed using six consecutive polymorphic microsatellite DNA markers that span an interval greater than 56 cM in region 2q34, where fibronectin, villin, and desmin map in close proximity (Fig. 1). CTLA4 is flanking the fibronectin gene locus on the proximal and D2S172 on the distal side (Fig. 1). Ninety-nine individuals of the GFD kindred were haplotyped (Fig. 2). Haplotype data are shown in Figure 2 for subfamilies of second generation individuals II-6 and II-14. Additional descendants of the other individuals in generation II were also haplotyped (data not shown), thus corroborating the haplotypes inferred for generations I and II.

Since in the presence of age-related penetrance the absence of disease cannot be determined with certainty in generations IV and V haplotype data are evaluated on the basis of an "affecteds-only" strategy, in which affected individuals are examined for cosegregation of haplotypes. It is apparent from Figure 2 that none of the haplotypes identified cosegregate with GFD. At best there is cosegregation in only 6 or 5 out of 12 affected individuals, for which haplotype data are available (see haplotypes depicted as a vertical hatched bar or a white bar, respectively, in Fig. 2.) If unaffected individuals of generation II are also taken into account, the absence of cosegregation is even more apparent. Therefore, there is complete *absence* of cosegregation between GFD and haplotypes extending from the proximal flanking marker CTLA4 to the distal flanking marker D2S172, thereby excluding fibronectin and the

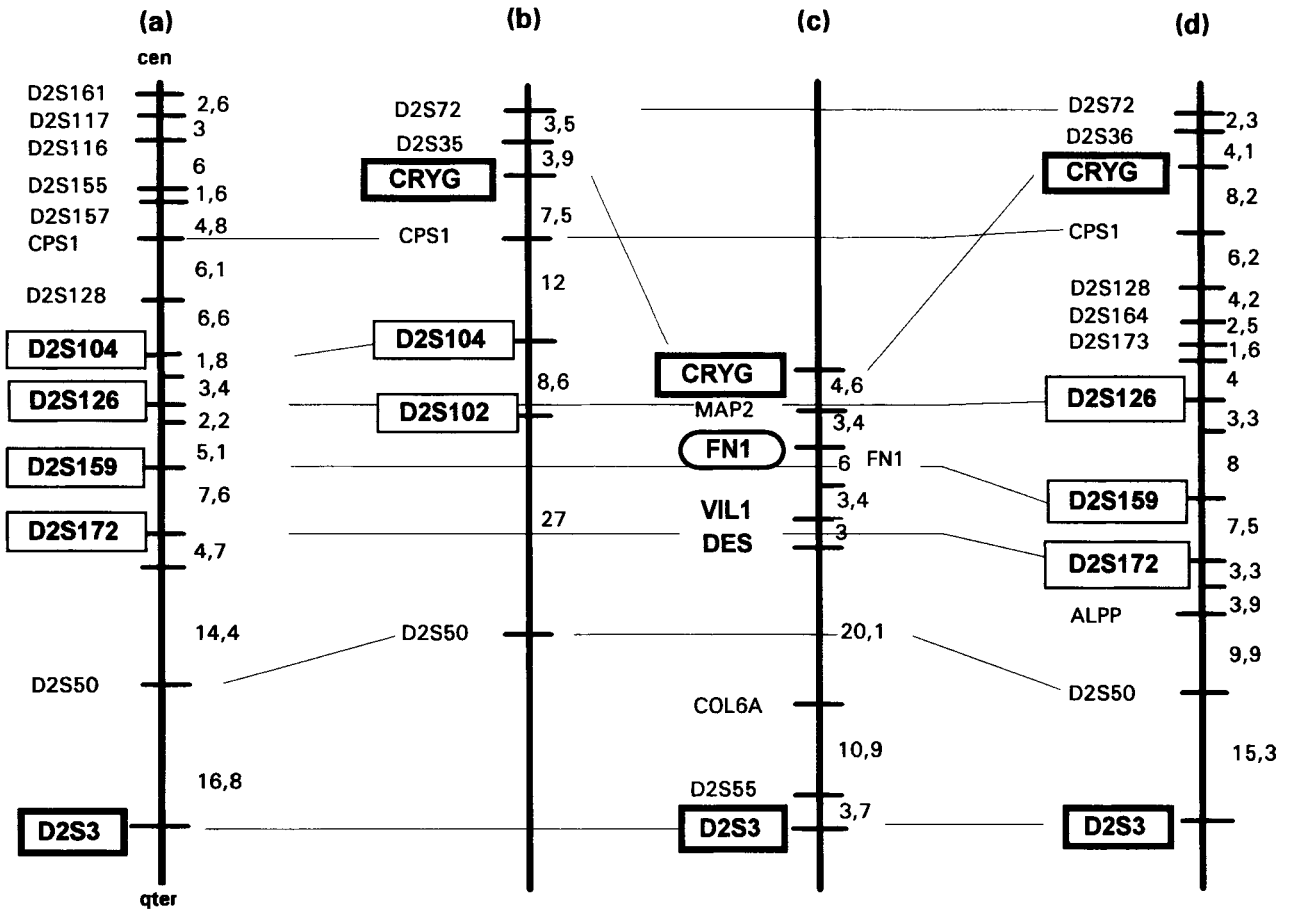

Fig. 1. Line-up of four genetic maps of polymorphic DNA markers in the fibronectin region 2q34 [a, Buetow et al., 1994; b, NIH/CEPH Collaborative Mapping Group, 1992; c, Shaw et al., 1993; d, Flint et al., 1994]. Maps are drawn to scale giving genetic distances between markers in cM. Maps a, c, and d are aligned for the most distal marker D2S3 (solid box), which is common to all three maps. Maps b and d are aligned for the proximal marker CRYG (solid box), which is common to both of these maps. Markers common to at least two maps are interconnected by thin lines. The map location of the fibronectin gene (FN1) is shown in an oval. Symbols for microsatellite markers used in haplotype analysis are given in thin-lined boxes. CTLA4 (not shown) maps to 2q33 and, therefore, represents a proximal flanking marker to fibronectin (FN1), which maps to 2q34 [Spurr et al. 1994]. The gene loci for villin (VIL1) and desmin (DES) map in close proximity to fibronectin (FN1).

neighbouring genes for villin and desmin from being the causative gene for GFD in this kindred.

DISCUSSION

Glomerular fibrillary deposits have been described in three different disease entities: amyloidosis, immunotactoid glomerulonephritis, and fibrillary glomerulonephritis. Recently, GFD has been distinguished from these disease entities as a fourth type of glomerulopathy by virtue of deposits that show strong immune reactivity to fibronectin [Strøm et al., 1995]. While for certain types of amyloidosis a genetic defect has been identified on a molecular basis [for review see Hildebrandt [1995]], no genetic defect has been described so far for GFD. Strong immunostaining with antibodies against plasma-derived fibronectin was a constant finding in the GFD families described [Strøm et al. 1995]. The recurrence of disease in one transplanted kidney in an individual of the family described here indicates the presence of a circulating factor, which could be identical with the deposited material or parts thereof or, alternatively, could interfere with the glomerular handling of the deposited material. We, therefore, examined the hypothesis that a genetic defect in the gene for fibronectin would be responsible for the disease.

The finding that the gene loci for fibronectin, villin, and desmin, the latter two of which are known to bind to fibronectin, were excluded from linkage to the GFD gene locus, excludes all three genes as causative for GFD. It will therefore have to be postulated that the genetic defect of GFD lies the gene for a different protein that by some unknown mechanism leads to intramembranous trapping of fibronectin in the glomerular basement membrane, and consequently to the clinical sequelae of GFD. We, therefore, are conducting additional candidate gene evaluation

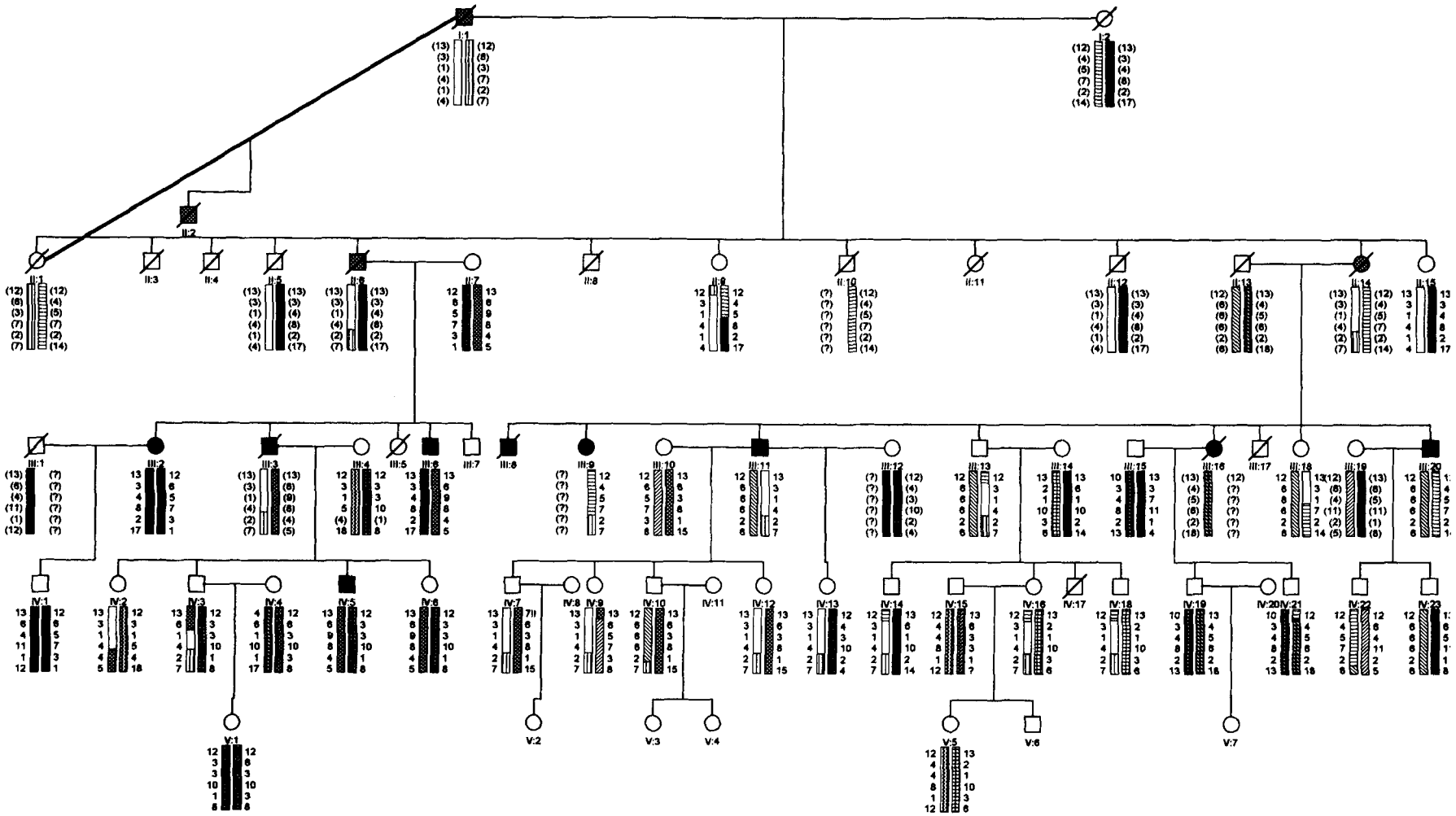

Fig. 2. Haplotype analysis with six consecutive markers (Fig. 1) of the fibronectin (FN1) region in subfamilies of second generation individuals II-6 and II-14 of the GFD pedigree. Beyond the second generation only the descendants of individuals II-6 and II-14 are shown for matters of space. Sibs are ordered from left to right in descending age. Additional descendants of the other individuals in generation II were also haplotyped (data not shown), thus corroborating the haplotypes inferred for generations I and II. In all, 99 individuals were haplotyped. The full pedigree was prepared by Gemperle et al. (unpublished). The diagnosis of GFD in nine affected individuals was based on strict criteria as described in the methods section. Four additional individuals (I-1, II-2, II-6, and II-14), who were most likely affected by GFD (as indicated by hatched symbols), were not available for clinical studies. However their clinical history was fully compatible with the affected status. Note that in the presence of age-dependent penetrance the absence of disease cannot be determined with certainty in generations IV and V, since median age is 27 years in generation IV and 8 years in generation V. Haplotypes are given for the genotypes of microsatellite markers CTLA4, D2S104, D2S126, D2S102, D2S159, and D2S172 (order from top down). Inferred genotypes are shown in parenthesis. Haplotypes are coded in different shadings in order to alleviate assessment of segregation. The paternal haplotype is drawn to the left, the maternal haplotype is drawn to the right. An apparent new mutation for marker CTLA4 was detected in individual IV:7. Note that there is complete *absence* of cosegregation between GFD and haplotypes extending between flanking markers CTLA4 to D2S172.

and are performing a total genome search by linkage analysis in order to genetically map the gene responsible for GFD.

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